Xylanase-Aided Chlorine Dioxide Bleaching of Bagasse Pulp to Reduce AOX Formation

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Xylanase pretreatment was used to improve the chlorine dioxide bleaching of bagasse pulp. The pulp was pretreated with xylanase, which was followed by a chlorine dioxide bleaching stage. The HexA content of the pulp and the AOX content of the bleaching effluent were measured using UV-Vis and GC-MS methods, respectively. The results showed that a good correlation occurred between HexA and kappa number. HexA content of the pulp decreased significantly after the xylanase pretreatment. The AOX content of the bleaching effluent decreased as HexA was removed from the pulp. It was found that AOX could be reduced by up to 29.8%, comparing XD_0 with a D_0 stage. Fourier transform infrared spectroscopy (FTIR) was employed to determine the breakage of chemical bonds in the pulp. It revealed that some lignin and hemicellulose were removed after xylanase treatment. The GC-MS results showed that some toxic chloride such as 2,4,6-trichlorophenol could be completely removed after xylanase pretreatment.

Keywords: Xylanase; Hexenuronic acid; AOX; FTIR; GC-MS

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INTRODUCTION

In the past two decades, the global pulp and paper industry has undergone great changes in response to environmental pressure. Chlorinated bleaching agents reacting with kraft pulp generate a complex mixture of degradation products, such as adsorbable organic halogens (AOX), which adversely impact the environment (Sharma *et al*. 2015). Meanwhile, AOX is regarded as an extremely important parameter to test and as an indicator of environmental influence (Lehtimaa *et al.* 2010). Rigorous environmental laws promote technological developments that would reduce chlorine from emissions of AOX in bleaching effluents. With this background, biotechnology surrounding the pulping process has been developed rapidly. Lots of studies have been conducted dealing with enzyme bleaching, including xylanase (Shatalov and Pereira 2007; Fillat and Roncero 2009), laccases, and two enzymes treated jointly (Aracri and Vidal 2011; Fonseca-Maldonado *et al.* 2014), which provided the possibility for industrial application in the pulp bleaching process.

Many studies have focused on the effect of xylanase pretreatment in the context of bio-bleaching. Some advantages of xylanase application are low capital investment, higher pulp brightness, reduction of bleaching chemicals [15 to 20%], and low AOX concentration in the final effluent. Xylanase use has saved on chemical costs for mills without changing the existing process. Sharma *et al*. (2015) observed that bio-bleaching, using xylanase-producing *Bacillus halodurans* FNP 135 through submerged (SmF) and solid state

fermentation (SSF), could result in 20% and 10% reductions in chlorine consumption, respectively. Singh and Dutt (2014) investigated the effect of enzyme treatment on wheat straw soda-AQ pulp by using xylanase from strains SH-1 NTCC-1163 (enzyme-A) and SH-2 NTCC-1164 (enzyme-B), and found that the AOX could be reduced by 38.75% and 36.25% in $OXAE_1DE_2P$ and $OXBE_1DE_2P$ bleaching processes, respectively. However, the data collected were still indistinct as to how xylanase pretreatment affected AOX and what changes occurred in the structure at the fiber surface and AOX components in XD_0 bleaching progress (Nie *et al.* 2015).

This research investigated pretreatment of bagasse kraft pulp by using xylanase to investigate the influence of xylanase pretreatment on AOX content in effluent. Therefore, this study explored the changes in the chemical groups on the surface of bagasse pulp by Fourier transform infrared (FTIR) spectroscopy. The species and concentration of chlorinated organic components in effluent were determined by gas chromatography-mass spectrometry (GC-MS). The results of this study have important potential implications for kraft pulp mills and the environment (Lehtimaa *et al.* 2010).

EXPERIMENTAL

Materials

The unbleached bagasse pulp was procured from a pulp mill situated in Guangxi, China. The brightness, Kappa number, viscosity, and HexA content of the initial pulp were 32.83 ±0.1% ISO, 15.02 ±0.05, 998 ±1 mL/g, and 14.7 ±0.1 mmol/kg (oven-dried pulp), respectively. The xylanase was produced by SUKAHAN Company (Shandong, China).

Methods

Xylanase pretreatment

The bagasse pulps were thoroughly mixed with the xylanase solution in a plastic bag. The enzymatic pretreatment conditions were as follows: 55 °C, 60 min, pH 7.0, and 10% pulp consistency. The xylanase pretreated pulps were completely washed and put in plastic bags. The D_0 stage was carried out at the condition of using 3% chlorine dioxide (percentages referring to oven-dried pulp), 10% pulp consistency, 65 °C, and pH within the range of 3.5 to 4.0 for 1 h after X stage.

Analysis of AOX and pulp properties

The AOX measurements were obtained using a Multi X 2500 halide analyzer (Jena, Germany). Micro coulomb titration method was used to calculate the content of AOX in effluent (Nie *et al.* 2013, 2014a,b). The functional group radical changes in pulp were detected by FTIR instrument, and species of chlorinated organic components in effluent were determined by GC-MS system after D_0 stage and XD_0 stage. Pulp brightness and kappa number were detected according to ISO 3688 and ISO 302, respectively.

Analysis of HexA content

The content of HexA in the pulp was detected by using spectrophotometric method (TAPPI Test Method T 282 om-13). The pulp was hydrolyzed by using mercury chloride solution and sodium acetate at 65 °C for 30 min. Absorbance was measured at 260 nm and 290 nm. The HexA content was calculated as,

$$C_{HexA} = 0.287\left(\frac{A_{260} - 1.2A_{290} \bullet V}{w}\right) \quad (1)$$

where C_{HexA} is the HexA molar fraction in the pulp expressed as (mol HexA /g dry pulp); A_{260} and A_{290} are absorbance values determined at 260 and 290 nm, respectively; V is hydrolysis solution volume in mL, and w is the pulp dry weight (g)

FTIR and GC-MS analysis

The pulp was washed with deionized water, subsequently air-dried, and cut into pieces small enough to fit through 120 mesh screens. Then samples were embedded in a KBr disk and detected at room temperature. Spectral data in the range of 3500 to 800 cm^{-1} were collected by employing a Bio-Rad FTS 6000 FT-IR spectrometer (Cambridge, MA) equipped with a MTEC 300 photoacoustic detector (Ames, IA) (Bjarnestad and Dahlman 2002).

Samples were analyzed on a GC-MS (GCMS-QP2010, Shimadzu, Japan) equipped with DB-5MS column (30 m x 0.32 mm, and 0.25 μm film thickness). The chromatographic control conditions were as follows: the flow rate of carrier gas (He) was 50 cm/s; the original column temperature was 65 °C (hold for 2 min) and was raised to 220 °C at a rate of 9 °C /min, and then the temperature was held for 20 min. The transfer-line temperature and injector was 300 °C. The injection volume was 1 μm, and the split ratio was 1:10. MS detected at the condition of voltage 1.05 kV, EI 70 eV, scan field 35 to 350 m/z, and ion source temperature 200 °C (Lei *et al.* 2007).

RESULTS AND DISCUSSION

Effect of HexA on Kappa Number During Chlorine Dioxide Bleaching

Figure 1 shows the correlation between HexA and Kappa number. The results were obtained after durations of 3, 5, 10, 15, 20, 30, and 60 min during the D_0 stage. The content of HexA decreased continuously in the chlorine dioxide bleaching process (Fig. 1). The results indicated that HexA had a negative effect on chemical bleaching agents. Kappa number is a combination of the contributions of residual lignin, HexA, and other chemical structures (Valls and Blanca Roncero 2013). In the bleaching process, a good correlation has been obtained between HexA and kappa number. Vuorinen *et al*. (1999) concluded that the amount of HexA removed correlated linearly with the reduction of kappa number. As shown in Fig. 1, the slopes between HexA and kappa number were similar during application of the different dosages of chlorine dioxide (1%, 3%, 5%). Parts of HexA may have been removed by the effect of oxidation on their double bonds, which also caused the reduction of Kappa number. Costa and Colodette (2007) also found that HexA was degraded indirectly in chlorine dioxide bleaching by hypochlorous acid and/or chlorine. At different chlorine dioxide dosage, the residual and consumed HexA in the reaction were also different. This demonstrated that HexA was able to react during the chlorine dioxide treatment and to consume parts of chemical bleaching agent, which was consistent with the later result that the HexA reacted with the oxidizer and generated AOX in the effluent.

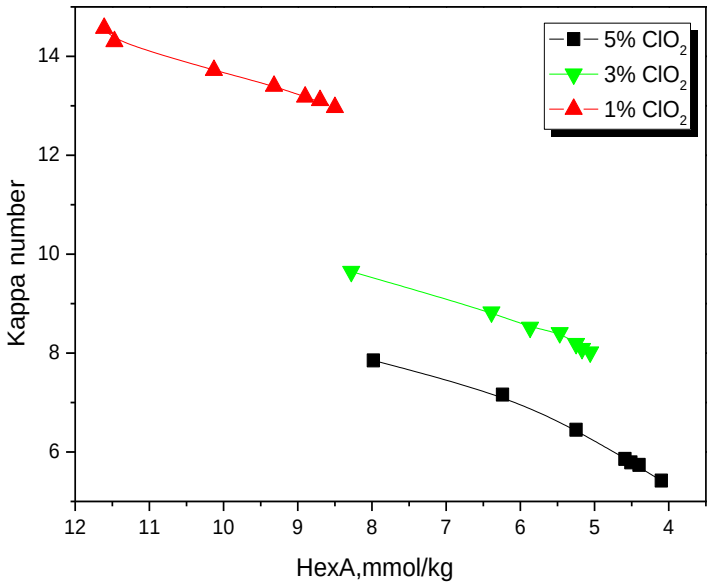

Fig. 1. Correlation between HexA content and kappa number

Effect of Xylanase Pretreatment on HexA, Kappa Number, and Bleachability

Figure 2 shows the results of xylanase pretreatment on HexA and kappa number. As can be seen, HexA decreased with the increasing of xylanase dosage. As HexA combined with xylan in the pulp, the reduction of HexA content may be caused by the removal of xylan. Moreover, because of the presence of -COOH and C = C in HexA, there was an adverse effect on bleaching and whiteness stability. This was similar to the report that the xylanase boosted HexA removal, which probably would through the elimination of xylan from the fiber surface (Shatalov and Pereira 2007; Shatalov and Pereira 2009). This result was consistent with a study showing that eucalyptus kraft fiber became fibrillar and rough with an occurrence of pores and flakes on the surface (Sharma *et al.* 2015). Meanwhile, the kappa number was shown to be a directly related process parameter.

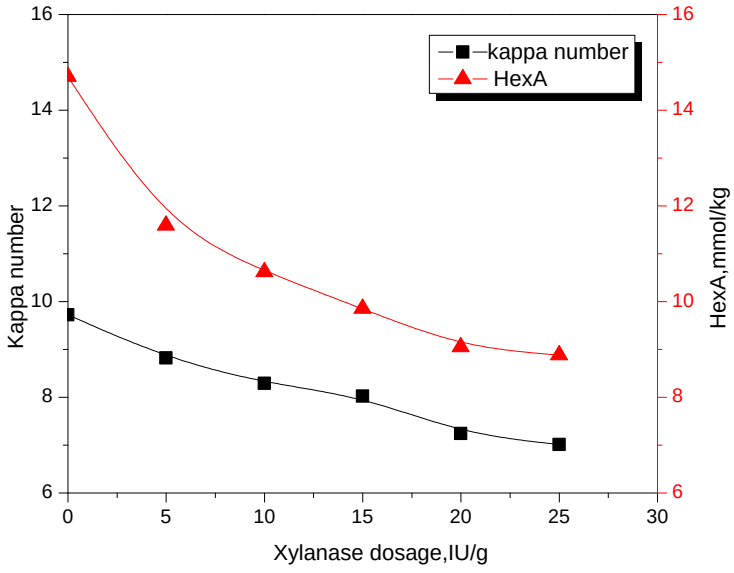

Fig. 2. Effect of xylanase pretreatment on HexA and kappa number

Figure 2 shows two trending lines that are surprisingly similar. As mentioned before, kappa number is a combination of the contributions of residual lignin, HexA, and other minor structures, so the drop of kappa number may be related to the removal of HexA. Moreover, another reason may be the extraction of LCC under xylanase pretreatment, which causes part of lignin to degrade. These results are similar with studies of LCC dissolved into effluent after xylanase pretreatment (Dai *et al.* 2014).

Figure 3 shows the correlation between xylanase dosage and pulp yield. The screened yield is an extremely important economic parameter for the pulp manufacturer. As expected, a minor reduction in the screened yield was observed as the dosage of xylanase increased. One reason may be the degradation of some hemicellulose and a little release of lignin after the xylanase pretreatment. This was similar with the result that such behavior was associated with the loss of carbohydrates, mainly the polysaccharides of low molecular weight (Almeida and Silva Júnior 2004).

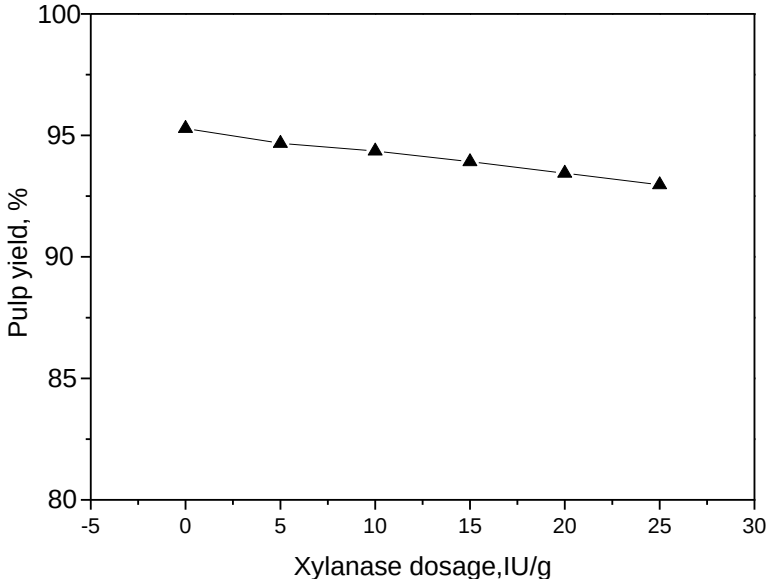

Fig. 3. Correlation between xylanase dosage and pulp yield

Effect of HexA Contents of the Pulp on AOX Formation

Figure 4 shows the correlation of HexA content of pulp on AOX. It is well known that AOX is one of the most commonly measured parameters to test environmental influence. Figure 4 shows that the AOX content decreased with the removal of HexA, within a certain concentration. This behavior was associated with HexA of pulp, which consumed chlorine dioxide and generated AOX in the effluent. A similar report indicated that alkali-cooked xylan reacted with chlorine dioxide and discharged AOX. About 2 to 4 μmol/g originated from HexA and corresponded to 9.0 to 16.3 mol of HexA groups (Magara *et al.* 2009). However, at the condition of 120 min, the AOX content almost had no change, meaning that only parts of HexA were removed in the chlorine dioxide bleaching process and affected the AOX content. Andreu and Vidal (2014) also obtained a similar conclusion that the HexA from the pulp generated unstable AOX in the D_O stage, and the removed HexA had a good linear relationship with the unstable AOX. Besides, the AOX content was 47.7 mg/L in a single D_O stage. However, the AOX content decreased to 32.8 mg/L after XD_O stage (X: 25IU/g, 120 min). The reduction of AOX was about

29.8%. This was a surprising result for pulp mills and the environment, which can cut down chlorine dioxide dosage and AOX emissions. Similar to our results, Gupta *et al.* (2012) showed that xylanase pretreatment was helpful in saving 23.1% of chlorine dioxide. Furthermore, xylanase pretreatment reduced the active chlorine multiple by 28.3% for XDEopDED process in a softwood kraft pulp run. The reduction in total ClO_2 consumption and AOX in bleach plant effluent was 15% (Scott *et al.* 1992).

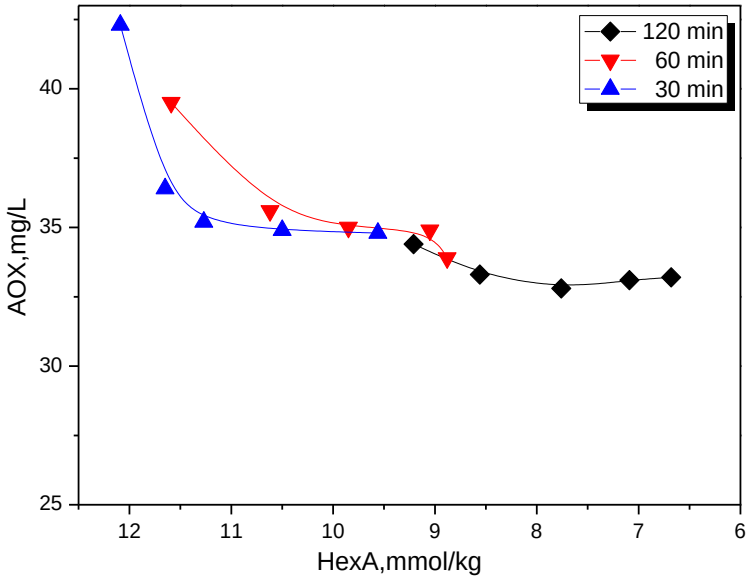

Fig. 4. Correlation between HexA and AOX content

Surface Chemistry of the Bagasse Pulps and GC-MS of Effluent

The changes in chemical groups on the surface of bagasse pulp were determined by FTIR. The corresponding spectra are shown in Figure 5. Comparing the two lines A and B, there was no evident change in the 2000 to 1400 cm^{-1} band. The remarkably changed areas were in the range of 1400 to 1000 cm^{-1}.

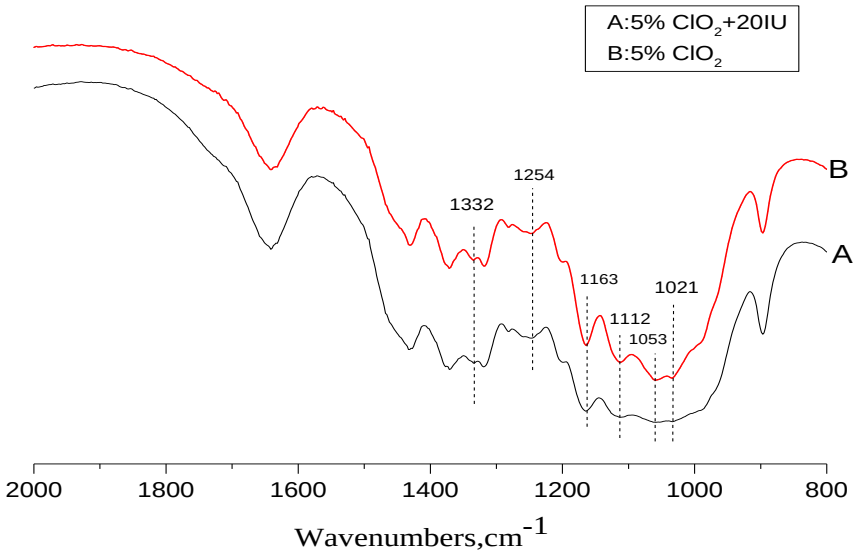

Fig. 5. FTIR spectra of bagasse pulp treated with D_0 process and XD_0 process

The band at 1332 cm^{-1} was assigned to lignin syringyl and condensation guaiacyl aromatic ring skeleton resonance, which changed little after xylanase pretreatment. The band at 1254 cm^{-1}, assigned as guaiacyl methoxy-C-O stretching vibration, became weakened in comparison to the untreated condition. Interestingly, there were some significant changes in the 1163 and 1112 cm^{-1} regions corresponding to C-O-C asymmetric stretching vibration and OH stretching vibration, respectively, meaning that those groups were degraded by xylanase treatment. The peak at 1049 and 1021 cm^{-1} was assigned to C-O-C stretching vibration, and C=O stretching vibration, which indicated some hemicellulose had been destroyed after xylanase pretreatment. Therefore, the main reaction center of xylanase treatment may have occurred on the position of hemicellulose linked with lignin. Coincidentally, the LCC and the C=O in HexA were located in this position.

Figures 6a and b show total ion chromatogram (TIC) of sulfate bagasse pulp wastewater with D_0 and XD_0 process, respectively. Table 1 shows the changes of species and concentration of chloride. The main source of organic environmental pollutants generated in the chlorine dioxide bleaching process had changed. The first remarkable result was that the xylanase pretreatment was very effective at removal of 1,1-dichloroacetone in XD_0 stage comparing with D_0 stage, for which a 42.4% reduction was obtained.

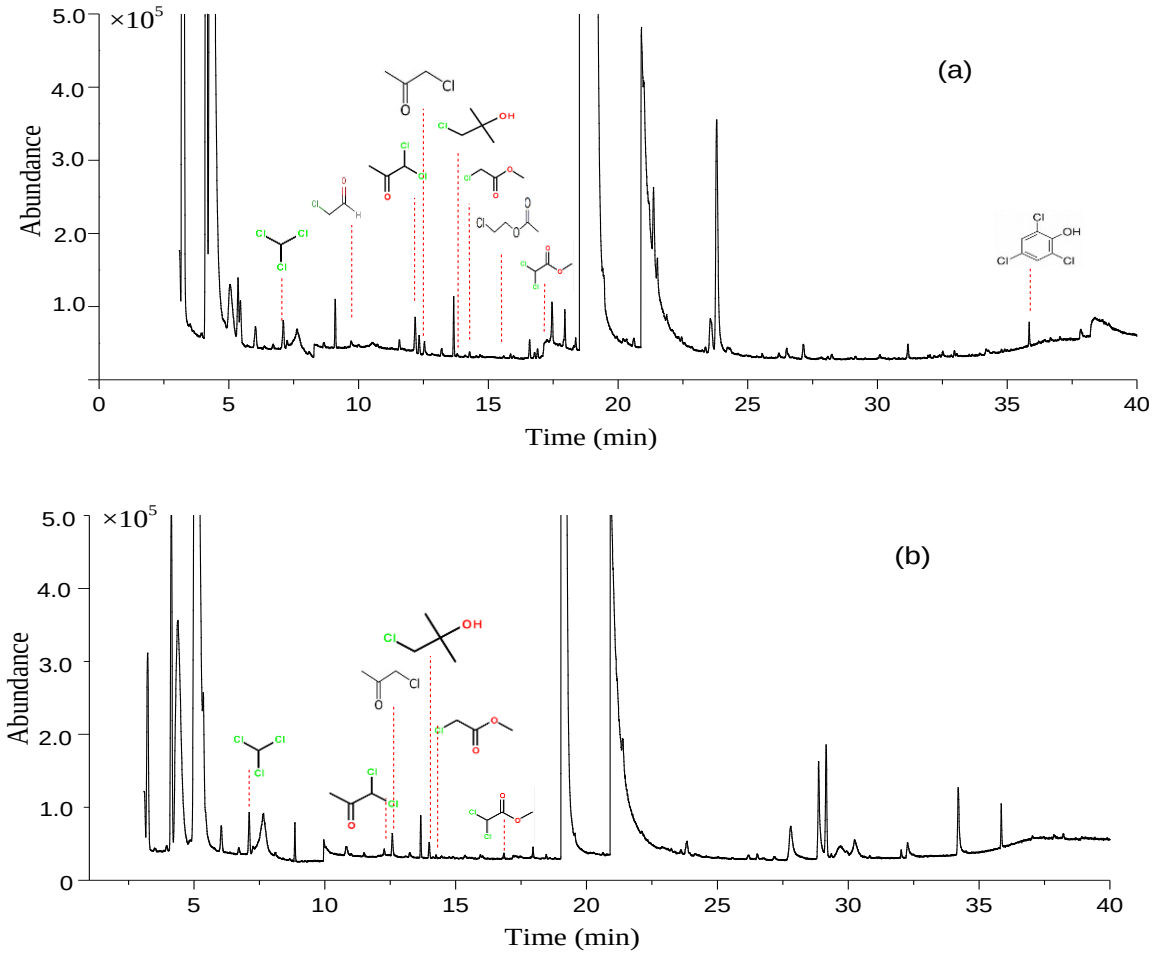

Fig. 6. TIC of sulfate bagasse pulp in wastewater a) D_0 stage and b) XD_0 stage

Furthermore, a surprising result was that the chloroacetaldehyde, 2-chloroethyl acetate, and 2,4,6-trichlorophenol have been removed completely through xylanase pretreatment. Those reduced or disappeared components were mainly related to the removal of a little LCC. Another explanation for this phenomenon was caused by the reduction of HexA, decreased by xylanase pretreatment, which caused lower HexA to react with chlorine dioxide and lead to the drop of those components. However, the content of chloroform, chloroacetone, 1-chloro-2-methyl-2-propanol, methyl chloroacetate, and methyl dichloroacetate were lower after xylanase pretreatment. The reason may be that some hemicellulose and LCC in bagasse pulp was degraded by xylanase; therefore, more lignin was exposed and reacted more easily with chlorine dioxide, which caused the increase in those compounds.

Table 1. Main Bleaching Substance in D_0 and XD_0 Stage

Remaining time (min)		Components	Relative content (%)	
D_0	XD_0		D_0	XD_0
12.33	12.27	1,1-dichloroacetone	6.1	3.3
9.72	-	Chloroacetaldehyde	1.2	0
15.59	-	2-Chloroethyl Acetate	0.4	0
36.67	-	2,4,6-Trichlorophenol	1.0	0
7.10	7.11	Chloroform	5.8	27.1
12.54	12.57	Chloroacetone	2.2	13.4
13.80	13.99	1-chloro-2-methyl-2- propanol	0.4	9.1
14.28	14.25	methyl chloroacetate	1.0	2.3
16.90	16.85	methyl dichloroacetate	1.0	3.4

As stated above, the cellulose surface of unbleached bagasse pulp was modified by the xylanase pretreatment, possibly as a consequence of removing surface compounds, including short-chain xylan, lignin, and non-fibrous material. Looking back at the relevant conclusions, the kappa number of bagasse pulp had decreased and bleachability had increased after the enzymatic pretreatment. Comparing the results of xylanase pretreatment with the single D_0 process, an organic chloride compound with a benzene ring, such as the toxic compound 2,4,6-trichlorophenol, can be removed completely, which indicated that a small amount of lignin disappeared. Therefore, it can be concluded that xylanase pretreatment before D_0 stage was beneficial to reduce lignin. These results were consistent with the previous results that kappa number decreased after xylanase pretreatment. It was known that HexA was generated from bagasse pulp during the kraft cooking stage. The HexA content decreased after xylanase pretreatment, which showed that xylanase-aided treatment could lower HexA content. A study has confirmed that HexA of low molecular weight was able to be oxidized during chlorine dioxide bleaching process (Magara *et al.* 2009). Furthermore, Bjorklund *et al.* (2004) also found that a part of unstable AOX was generated from HexA. Those findings may highlight that the reduced chlorides of AOX were likely to be the products which HexA reacted with oxidant and cracked during the chlorine dioxide bleaching stage. Therefore, the reaction mechanism may be that enzyme treatment modified and removed some xylan and LCC in the pulp, which caused the reduction of AOX in the subsequent D_0 bleaching stage.

CONCLUSIONS

1. Pretreatment of pulp with xylanase was found to reduce the HexA from bagasse kraft pulp and hence reduce the formation of AOX that was generated during the subsequent chlorine dioxide bleaching of the pulp. HexA is closely related to AOX formation, as well to kappa number and pulp brightness.
2. It was found that AOX could be reduced by up to 29.8% comparing a XD_0 treatment with a D_0 bleaching stage. It was also found that the chlorophenol compounds could be completely removed after xylanase pretreatment.

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